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**STUDIES OF COHESIN FUNCTIONS IN  
THE YEAST AND HUMAN DNA DAMAGE RESPONSE**

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*To the memory  
of my mother*

## ABSTRACT

Maintaining genome stability is critical to cell survival and normal cell growth and most human cancers display some form of genome instability. Genome instability is caused by multiple reasons and the ability to properly recognize, signal and subsequently repair DNA damages is crucial. DNA double-strand breaks (DSBs) are considered as the most toxic type of DNA lesion and therefore the ability to accurately repair these breaks are of outmost importance.

The cohesin complex is a large DNA-binding complex with numerous functions vital for maintaining genome integrity. The canonical role of the cohesin complex is to mediate cohesion between the sister chromatids from the time they are generated to their separation in mitosis. However, cohesin has over the years been assigned with additional functions independent on cohesion, such as regulation of gene transcription, DSB repair and activation of DNA damage checkpoints.

We have investigated the role of cohesin and its loading partner NIPBL, in the cellular responses to DSBs, using human cell cultures and budding yeast as model systems.

There are two main mechanisms used to repair DSBs, homologous recombination (HR) and nonhomologous end joining (NHEJ). More recently, a new “alternative” pathway for DSB repair has emerged, termed alternative end joining (A-EJ). By studying B-cells derived from patients with Cornelia de Lange Syndrome, we have observed a strong correlation between heterozygous loss-of-function mutations in the *NIPBL* gene and a shift towards the use of the microhomology-based A-EJ mechanism for DSB repair during class switch recombination. Furthermore, the early recruitment of 53BP1 to DSBs was reduced in the NIPBL-deficient patient cells. Our results suggest that NIPBL plays an important role for NHEJ, potentially by regulating DNA end resection.

In budding yeast postreplicative cells, the cohesion is reactivated in response to a DSB. This reactivation includes additional Scc2-dependent loading of cohesin to the region around the DSB and formation of new cohesion, both proximal to the DSB and on undamaged areas of the genome. This phenomenon is known as damage-induced (DI) cohesion. By analyzing the role of DNA polymerase  $\eta$  in DI cohesion we discovered that establishment of DI cohesion at the vicinity of a DSB and on undamaged chromosomes, genome wide, are regulated differently. We concluded this based on our finding that Pol $\eta$  is required for genome-wide DI cohesion while it is dispensable for S phase cohesion and DSB-proximal cohesion. Cohesion establishment, both during S phase and following a DSB, depend on the acetylation activity of the highly conserved acetyltransferase Eco1. Using *in vitro* studies, we found that Pol $\eta$  is an Eco1 substrate. In addition, we provide results suggesting that Eco1 acetylation of Pol $\eta$  regulates its activity in DI-cohesion.

All together, these studies highlight the importance of cohesin, and its regulators, for genome stability. Future investigations, aimed at addressing the different mechanisms by which cohesin functions in the DNA damage response will most likely advance our understanding of how genome stability is maintained.

## LIST OF PUBLICATIONS

This thesis is based on the following articles and manuscripts. They will be referred to in the text by their roman numerals.

- I. **Enervald, E.\***, Du, L.\*, Visnes, T., Björkman, A., Lindgren, E., Wincent, J., Borck, G., Colleaux, L., Cormier-Daire, V., van Gent, D., Pie, J., Puisac, B., de Miranda, N., Kracker, S., Hammarström, L., Villartay J.P., Durandy, A., Jacqueline Schoumans, J., Ström, L. and Pan-Hammarström, Q.  
A regulatory role for the cohesin loader NIPBL in non homologous end joining during immunoglobulin class switch recombination.  
*Accepted for publication in J. Exp. Med. 2013*  
\* Equal contribution
- II. **Enervald, E.\***, Lindgren, E.\*., Katou, Y., Shirahige, K. and Ström, L.  
Importance of Polη for damage-induced cohesion reveals differential regulation of cohesion establishment at the break site and genome-wide.  
*PLoS Genet. 2013;9(1)*  
\* Equal contribution
- III. **Enervald, E**, Palmgren, C., Rutishauser, D. and Ström, L.  
Acetylation of DNA Polymerase η by Eco1 regulates its function in genome-wide damage-induced cohesion.  
*Manuscript*

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## LIST OF ABBREVIATIONS

A-EJ	Alternative end joining
AID	Activation induced cytidine deaminase
ATM	Ataxia telangiectasia mutated
BLM	Bloom
BRCA1,2	Breast cancer antigen 1,2
CARs	Cohesin associated regions
CDK	Cyclin dependent kinase
CdLS	Cornelia de Lange syndrome
ChIP	Chromatin immunoprecipitation
CIN	Chromosome instabilities
C-NHEJ	Classical- nonhomologous end joining
CSR	Class switch recombination
CTCF	CCCTC-binding factor required for transcriptional repression
CtIP	CtIP-interacting protein
Chk1,2	Check point kinase 1,2
DI cohesion	Damage-induced cohesion
D-loop	Displacement loop
DNA Lig4	DNA ligase 4
DNA PKcs	DNA dependent protein kinase catalytic subunit
Dnl4	DNA ligase 4
DSB	Double strand break
Eco1	Establishment of cohesion 1
ESCO1,2	Establishment of cohesion 1,2
Eso1	Sister chromatid cohesion protein/DNA polymerase eta
Exo1	Exonuclease 1
$\gamma$ -IR	Gamma irradiation
H2A	Histone 2A
HDAC8	Histone deacetylase 8
HJ	Holliday junction
Hos1	Hda one similar 1
HR	Homologous recombination
Ig	Immunoglobulin
IR	Ionizing radiation
Ku	Ku70-Ku80 complex
Lif1	Ligase interacting factor 1
MDC1	Mediator of DNA damage checkpoint protein
Mec1	Mitosis entry checkpoint
Mre11	Meiotic recombination 11 homolog A
NBS1	Nijmegen breakage syndrome 1
Nej1	Nonhomologous end joining defective 1
NHEJ	Nonhomologous end joining
NIPBL	Nipped-B-like
PARP 1	Poly (ADP-ribose) polymerase 1
PCNA	Proliferating cell nuclear antigen
PDS5	Precocious dissociation of sisters 5
PIP	PCNA-interacting peptide
Pol	Polymerase



Rad	Radiation sensitive
RNAi	RNA interference
RBS	Roberts syndrome
RPA	Replication protein A
SA	Stromal antigen
Sae2	Sumo activating enzyme subunit 2
Sgs1	Small growth suppressor 1
SCC	Sister chromatid cohesion
SMC	Structural of maintenance of chromosomes
S region	Switch region in the Ig locus
ssDNA	single stranded DNA
Tel1	Telomere maintenance 1
TLS	Translesion DNA synthesis
UBZ	Ubiquitin-binding zinc-domain
UV	Ultraviolet
V(D)J	Variable (diversity) joining
WAPL	Wings a part like
WRN	Werner
XLF	XRCC4-like factor
XP-V	Xeroderma pigmentosum variant
XRCC	X-ray repair cross-complementing protein
Xrs2	X-ray sensitivity 2
53BP1	p53 binding protein 1



# 1. INTRODUCTION

The research presented in this thesis aimed towards elucidating the role of cohesin in the DNA damage response. In doing so, either *Saccharomyces cerevisiae* (budding yeast) or human cell cultures have been used as models. Genes and proteins discussed in general will be presented with the yeast term and the human term separated with a /.

## 1. THE CELL CYCLE

All living cells depend on the ability to duplicate. The event in which a cell faithfully divides in two is known as the cell cycle. A prerequisite for cell division is that the genetic material (DNA) has been accurately replicated, generating two identical copies. The cell cycle is divided into four phases (Fig. 1). The replication of the genetic material occurs in the synthesis (S) phase and the segregation of the two DNA copies takes place during mitosis (M). S and M phases are temporally separated by two gaps (G), known as G1 and G2. During the G1 and G2 phases the cells grow, mature and prepare for S and M phases, respectively (Hartwell and Weinert 1989). In eukaryotic cells, the cell cycle is controlled by cyclin-dependent kinases (CDKs) (Nurse, Masui et al. 1998) and checkpoints that arrest cells during the cell cycle to avoid events occurring out of order, potentially leading to DNA damage or cell death (Hartwell and Weinert 1989).

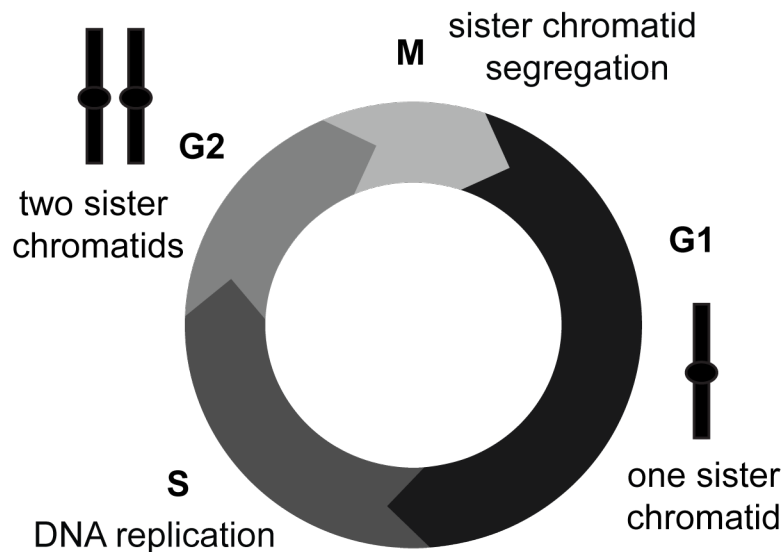


Figure 1. The cell cycle

## 2. REPAIR OF DNA DOUBLE STRAND BREAKS

DNA double-strand breaks (DSBs) are considered being the most toxic type of DNA lesion and the ability to accurately repair these breaks is essential for maintaining genome stability. If left un- or mis-repaired, DSBs can result in large deletions or genome rearrangements that ultimately may lead to carcinogenesis (Jackson and Bartek 2009). DSBs can arise following exposure to exogenous agents such as ionizing radiation (IR), resulting from radioactive decay from heavy metals or from radiotherapy

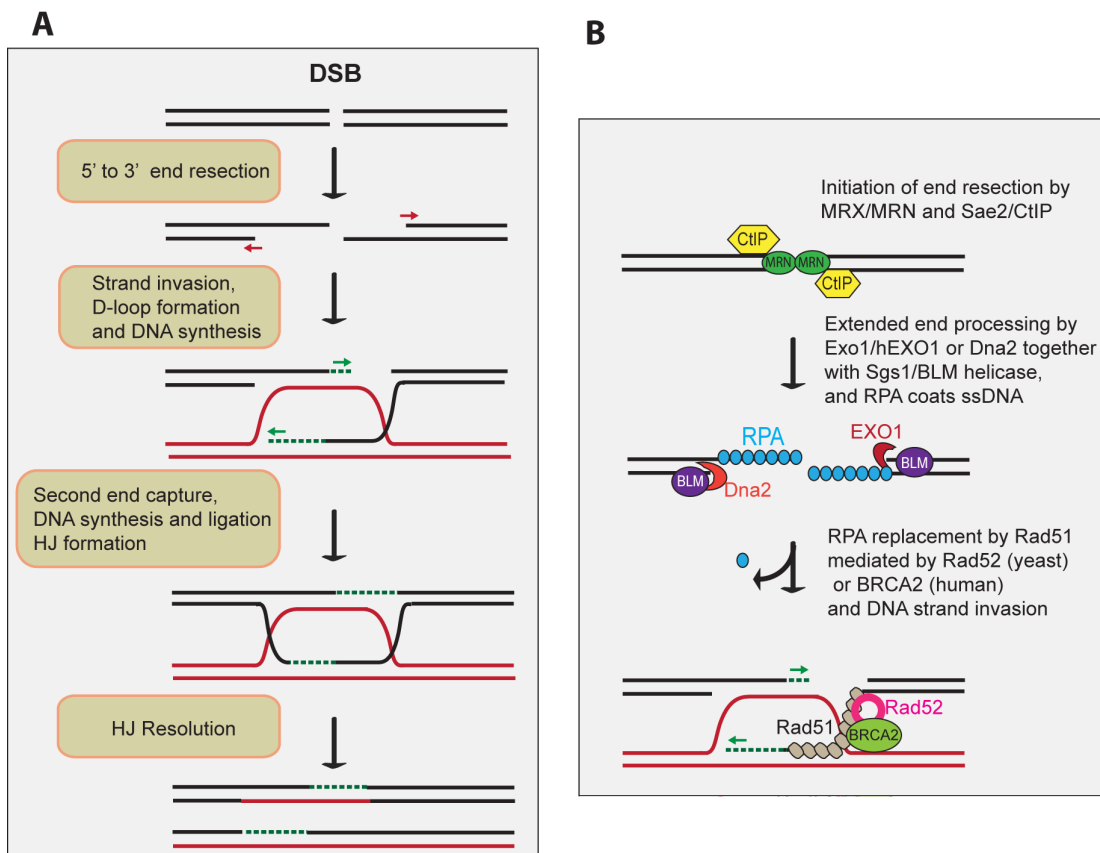
when treating certain cancers (Ward 1988). DSBs can also arise from harmful endogenous agents, such as reactive oxygen species generated as by-products from oxidative respiration. Replication stress caused by stalled replication forks is however the major endogenous source of DSBs (Pfeiffer, Goedecke et al. 2000). Interestingly, DSBs are purposefully and specifically induced during some cellular processes. Meiosis, V(D)J recombination, class switch recombination (CSR) and mating-type switch in yeast are all examples where induction of DSBs are programmed by the cell. Nevertheless, the repair of such DSBs is equally important (Pfeiffer, Goedecke et al. 2000).

There are two main mechanisms used to repair DSBs, homologous recombination (HR) and nonhomologous end joining (NHEJ). More recently, a new “alternative” pathway for DSB repair has emerged, termed alternative end joining (A-EJ). In the following sections I will describe the basic mechanisms of HR, NHEJ and A-EJ, and present an overview of what is known about DSB sensing and signaling and how the cell regulates the choice between the different repair pathways.

## 2.1 Mechanisms of repairing DSBs

### 2.1.1 Homologous Recombination

Among the DSB repair pathways, HR is considered the most accurate repair mechanism and is often referred to as an error-free pathway. By utilizing an undamaged homologous sequence as a template for the repair, HR guarantees high

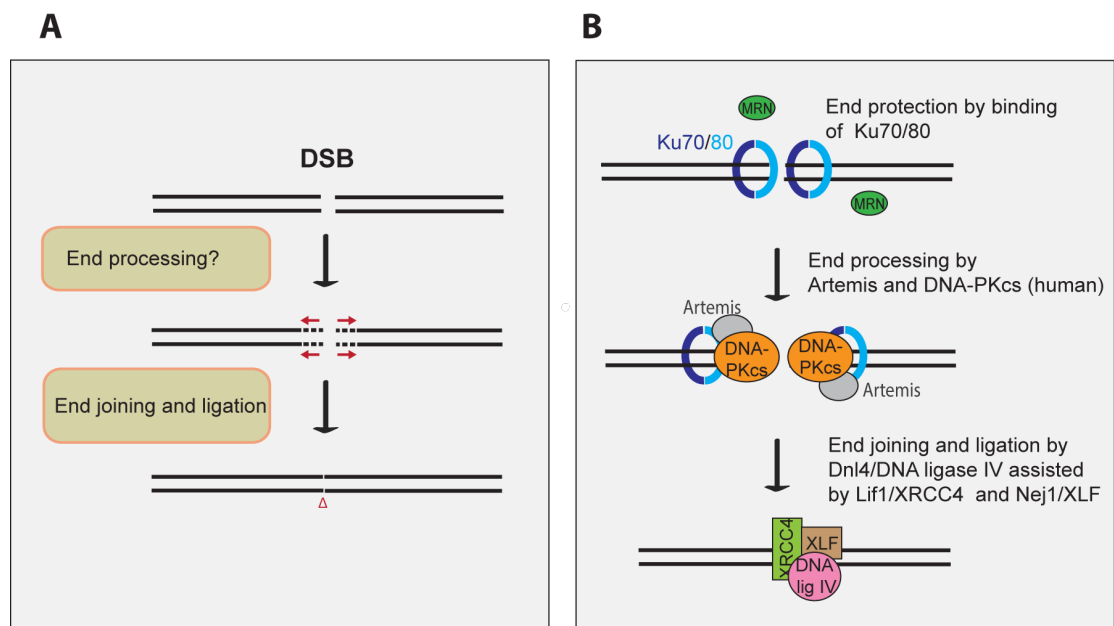


**Figure 2. Homologous recombination** **A.** schematic illustration of the sequential steps in HR **B.** detailed view on the initial steps in HR.

fidelity DSB repair. The HR repair pathway is initiated by a 5' to 3' end resection producing 3' single-stranded DNA (ssDNA) overhangs (Fig. 2). Resection of the DSB ends has been suggested to function by a two-step mechanism where the Mre11-Rad50-Xrs2/NBS1 (MRX/MRN) complex together with the 5'-3' exonuclease Sae2/CtIP participate in the initial end resection (Mimitou and Symington 2008; Clerici, Mantiero et al. 2005; Sartori, Lukas et al. 2007). For extensive resection, more processive exonucleases are recruited, either Exo1/hEXO1 or Dna2, together with the Sgs1/BLM helicase (Mimitou and Symington 2008; Zhu, Chung et al. 2008; Nimonkar, Ozsoy et al. 2008). Following end resection, RPA immediately covers the ssDNA to protect the ends from being degraded and prevent formation of secondary structures. RPA is further replaced by the Rad51 recombinase, a process that is carried out with the assistance of recombinase accessory proteins, also called “mediator” proteins. In humans, BRCA2 has been shown to perform this function (Jensen, Carreira et al.). In yeast, BRCA2 is not present and Rad52 mediates the switch (Sung 1997). The Rad51-DNA nucleoprotein filament catalyses the strand invasion, where one of the ends invades an intact duplex DNA molecule, forming a displacement (D-) loop. The invading 3' DNA end and the second 3' end are both extended by DNA synthesis. Rad52 mediates the capture of the second 3' end to enable joining of the two ends and ligation of the two ends generates a Holliday junction (HJ) intermediate. Resolution of the HJ structure leads to repair of the break and results in either crossover or non-crossover products (for review; San Filippo, Sung et al. 2008). There are other types of HR models, including synthesis-dependent strand annealing and break-induced replication. These two models will not be discussed here but for a review see (Paques and Haber 1999).

### 2.1.2 Nonhomologous End Joining

The NHEJ repair pathway is considered to be the most straightforward process for repairing DSBs. By ligation of two DNA ends, NHEJ is mechanistically relatively simple. NHEJ only works with high fidelity if the DNA ends are compatible. When the

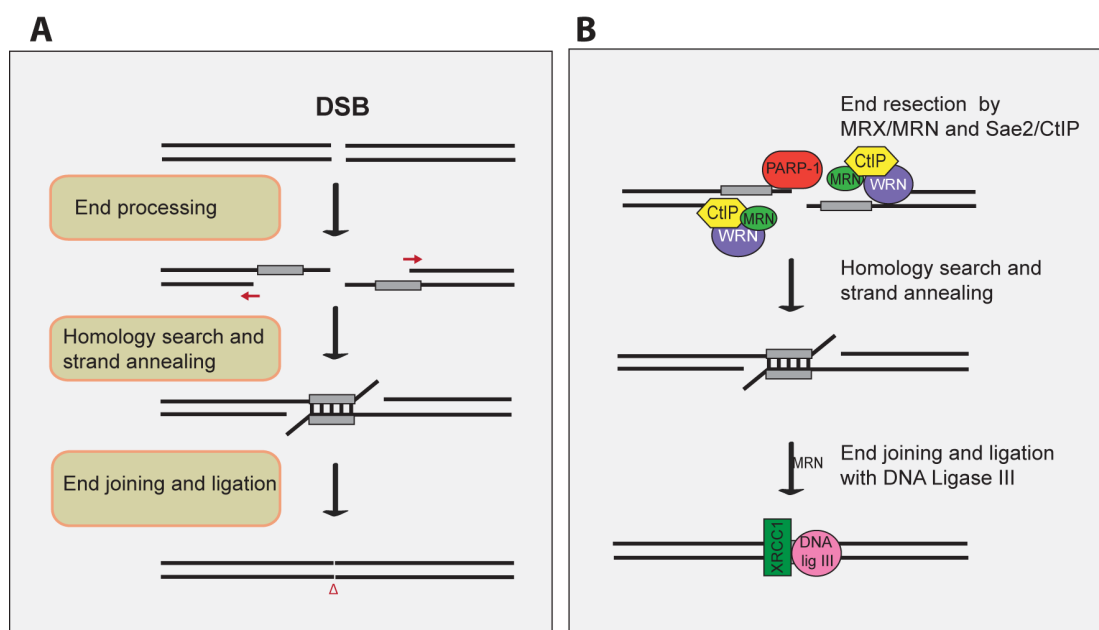


**Figure 3. Nonhomologous end joining** A. schematic illustration of the sequential steps in NHEJ B. detailed view on the steps in NHEJ.

ends are damaged and incompatible end processing is required which inevitably will result in loss of genetic material and NHEJ is indeed known to be error-prone. (Lieber, Gu et al. 2010). The first proteins in this pathway to bind DNA ends is Ku70-Ku80 (Ku), a heterodimer with a strong affinity for loose DNA ends (Fig. 3). Binding of Ku has a protective role on the DNA ends and is also necessary for recruitment of a number of processing factors (Lieber 2010). In mammalian cells, Ku interacts with DNA-PKcs and together they cooperate to synapse the two DNA ends (DeFazio, Stansel et al. 2002). In yeast however, no DNA-PKcs ortholog has been found. Depending on the structure of the DNA ends, end processing can be required prior to ligation. The nuclease Artemis is recruited and together with DNA-PKcs the DNA ends are trimmed (Ma, Schwarz et al. 2005). Finally, the ends are ligated by the Dnl4/DNA ligase IV, assisted by its cofactors Lif1/XRCC4 and Nej1/XLF (also called Cernunnos) (Nick McElhinny, Snowden et al. 2000; Critchlow, Bowater et al. 1997; For review see; Lieber 2010)

### 2.1.3 Alternative End Joining

One of the first descriptions of A-EJ came with the observation that yeast cells deficient in classical NHEJ (C-NHEJ) factors were still able to ligate DNA ends. Sequence analysis over the break showed deletions, larger than those repaired by C-NHEJ (Boulton and Jackson 1996). Since then, numerous of publications have started to elucidate the mechanism of A-EJ. Nevertheless it is still poorly understood. The kinetics of break repair by A-EJ appears slower to be than C-NHEJ (DiBiase, Zeng et al. 2000; Wang, Wu et al. 2006). The mechanism of A-EJ involves annealing of short homologous sequences (microhomologies), assisting in the ligation of the two ends. A-EJ is error-prone and involved in large deletions and chromosomal translocations (Simsek and Jasin 2010; Guirouilh-Barbat, Huck et al. 2004; Weinstock, Brunet et al. 2007). A-EJ has so far best been described in CSR, which could be explained by the high frequency of short repetitive sequences in these regions (Yan, Boboila et al. 2007). In the current model, A-EJ is initiated by 5' to 3' end-resection to expose complementary microhomologies (Fig. 4). Like HR, MRN and CtIP participate in the



**Figure 4. Alternative end joining** A. schematic illustration of the sequential steps in A-EJ B. Factors implicated in A-EJ mechanism

end resection (Deriano, Stracker et al. 2009; Xie, Kwok et al. 2009; Zhuang, Jiang et al. 2009; Cheng, Barboule et al. 2011; Zhang and Jasin 2010). Importantly, while HR requires longer ssDNA for efficient repair, less than 50 nt resection is sufficient for A-EJ (Grabarz, Barascu et al. 2012). DNA strand annealing of microhomologies (generally 2-8 nucleotides) tethers the DNA ends and creates branched intermediate structures. Ligation of the two ends appears to depend on DNA ligase III (Wang, Rosidi et al. 2005). Resolving the structure results in nucleotide deletions at the repair joint. Little is known about the factors that mediate A-EJ but XRCC1 and WRN helicase have been implicated in this process (Audebert, Salles et al. 2004; Sallmyr, Tomkinson et al. 2008). Additionally, A-EJ mechanism seems to depend on PARP-1 binding to DNA ends (Wang, Wu et al. 2006).

## 2. 2 Regulation of DNA Double Strand break repair

### 2.2.1 DSB recognition and checkpoint activation

How the cell responds to DNA damage depends on many aspects, but usually damage responses follows a common general program to translate the signal of damaged DNA into the appropriate downstream effect (Jackson and Bartek 2009). The damage response normally takes place in the following order; (i) DNA damage sensors, recognize abnormally structured DNA and initiate a damage response (ii) recruitment of mediators, that transmit the signal to (iii) transducers, factors that amplify and pass along the signal to (iv) effectors, that act in the cellular responses to the damage i.e. repair, apoptosis, and transcription (Fig 5; Jackson and Bartek 2009). In both yeast and human, a DSB is recognized by the MRX/MRN complex and Ku independently (Lisby and Rothstein 2009). Depending on the choice of repair pathway, either HR or NHEJ will be initiated. Regardless of which, the MRX/MRN complex activates DNA damage checkpoint by recruiting the checkpoint kinase Tel1/ATM. Tel1/ATM rapidly phosphorylates the histone variant H2AX (H2A in yeast) around the break. The key function of phosphorylation of H2AX (or H2A) is to provide a high-affinity binding

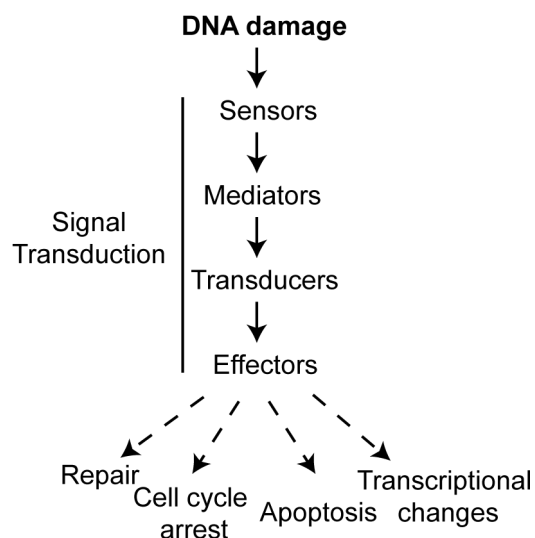


Figure 5 Model for the DNA damage response

platform for an assembly of damage response factors. In higher eukaryotes, the mediator protein MDC1 is recruited to phosphorylated H2AX ( $\gamma$ H2AX) and is believed to function as a molecular bridge between ATM, H2AX and the MRN complex, generating a feedback loop to amplify the  $\gamma$ H2AX signal. MDC1 is considered a master organizer of protein assembly at the damage site. A series of chromatin remodeling takes place leading to the recruitment of the downstream factors 53BP1 and BRCA1 (Sobhian, Shao et al. 2007; Huyen, Zgheib et al. 2004). In yeast, a

MDC1 ortholog has so far not been found. Instead, phosphorylated H2A facilitates binding of Rad9, the budding yeast ortholog of 53BP1, directly (Bekker-Jensen and Mailand 2010).

### **2.2.2 Regulating the DSB repair pathway choice**

Choosing the appropriate repair mechanism is crucial for efficient and faithful DSB repair. Though it is not fully understood how this choice is made, there are key steps and factors known to occur during the process.

Cell cycle phase is a key determinant in the choice of DSB repair pathway. While NHEJ is active throughout the cell cycle, HR is restricted to the S and G2 phases of the cell cycle when a sister chromatid is available as a template for the repair. By regulating DNA resection within the cell cycle, the commitment to HR is coordinated with DNA replication to occur primarily in S and G2 cells. By that, the initiation of end resection is a contributing key step in the choice of DSB repair pathway (Huertas 2010). Ku on the other hand, is believed to work as a barrier for end resection and displacement of Ku from the DSB is therefore crucial for efficient commitment to HR (Langerak, Mejia-Ramirez et al. 2011). However, once resection has started, Ku has very poor affinity for the DNA ends, further suppressing NHEJ. The balance between end resection (by MRN and CtIP) and end protection (by Ku) directly affects the fate of a DSB. 53BP1 and BRCA1 are other factors with emerging roles in steering repair towards NHEJ or HR, respectively (Chapman, Taylor et al.). The means by which 53BP1 mediates NHEJ is not completely clear but it has been shown to have an inhibitory effect on DSB resection (Bothmer, Robbiani et al. 2010). BRCA1 has been suggested to antagonize 53BP1 to permit end resection (Cao, Xu et al. 2009; Bunting, Callen et al. 2010). It is evident that there is a competition between NHEJ and HR (at least in higher eukaryotes) following DSB damage, where protein abundance and kinetics play an important role in the balance between the repair pathways.

The nature of the DSB is also of importance in the choice of repair mechanism. Programmed, endonuclease created DSBs, e.g. those induced during V(D)J recombination and CSR generate compatible ends and are almost exclusively repaired by NHEJ (Dudley, Chaudhuri et al. 2005).

The highly mutagenic nature of A-EJ makes it unlikely to be the pathway of choice when HR or NHEJ are viable options. However, growing evidence suggest that A-EJ is a robust pathway and potentially compete with both HR and NHEJ. A-EJ shares the initial end resection step with HR. For full commitment to HR, extended resection (>200 nt) is required (Rubnitz and Subramani 1984; Liskay, Letsou et al. 1987). An inefficient resection, not compatible with HR, might result in A-EJ events. This is seen in HR mutants with resected DNA ends (e.g. BRCA2), where Rad51 filament formation is disrupted, resulting in a shift towards the A-EJ pathway (Varela, Klijn et al. 2010). Increased usage of microhomologies has also been observed in cells deficient of C-NHEJ factors (e.g. Ku80 and XRCC4), indicating that the repair mode is also shifted towards A-EJ when C-NHEJ is dysfunctional (Kabotyanski, Gomelsky et al. 1998; Simsek and Jasin 2010; Guirouilh-Barbat, Huck et al. 2004). Ku has been shown to directly repress repair by A-EJ (Guirouilh-Barbat, Huck et al. 2004; Wang, Wu et al.



2006; Weinstock, Brunet et al. 2007; Fattah, Lee et al. 2010). PARP-1 has on the other hand been suggested to bind DNA ends in direct competition with Ku (Wang, Wu et al. 2006; Cheng, Barboule et al. 2011).

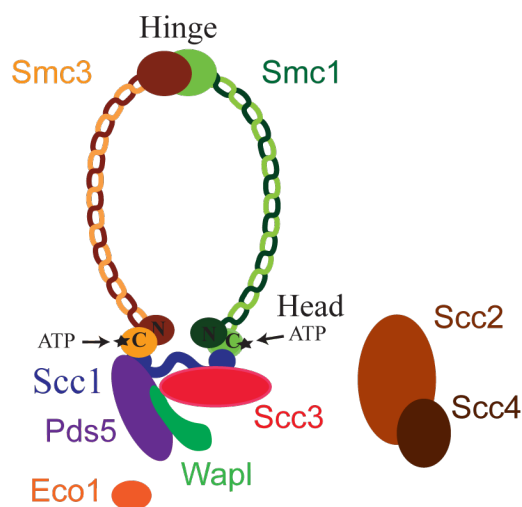
### 3. THE COHESIN COMPLEX

In 1987, Koshland and Hartwell published an investigation where they analyzed the topology of mitotic chromosomes. Their observations suggested that a protein or protein complex must serve the function of holding sister chromatids together until their separation in anaphase. They wrote; “Thus it is reasonable to postulate the existence of one or more interesting proteins that function to hold sister chromatids together” (Koshland and Hartwell 1987). 10 years later, Koshland’s and Nasmyth’s groups identified the protein complex that serves this function, namely the cohesin complex (Guacci, Koshland et al. 1997; Michaelis, Ciosk et al. 1997). Since then, several regulatory factors have been identified, the mechanism behind sister chromatid cohesion has become clearer and light has been shed on the undeniable importance of the cohesin complex for genome stability.

In the subsequent sections I will present the composition and molecular structure of the cohesin complex, as well as discuss the regulation of cohesin deposition and positioning onto chromatin. I will further present information regarding various cohesin functions in the DNA damage response.

#### 3.1. Cohesin structure and composition

The cohesin complex is a multisubunit protein complex (Table 1, Fig. 6). Cohesin belongs to the family of “structural maintenance of chromosomes” (SMC) protein complexes. Condensin and the Smc5/6 complex are the other two members of this family of large protein complexes. Common among the family members is that a pair of SMC proteins composes the core. The core of the cohesin complex is a heterodimer of Smc1 and Smc3 and like the other SMC proteins, Smc1 and Smc3 are elongated rod-shaped molecules. During the folding process each SMC molecule will bend over itself forming a “hinge” domain in one end while the C-and N-termini will come close to each other forming a globular ATPase “head” domain at the other end (Haering, Lowe et al. 2002; Hirano 2002). The Smc1 and Smc3 subunits are known to interact directly via their “hinge” domains, creating a V-shaped Smc1–Smc3 heterodimer. The Smc1 and Smc3 subunits are at their “head” ends connected via a subunit called Scc1/RAD21 that belongs to the kleisin protein family (Haering, Lowe et al. 2002).



*Figure 6. The cohesin complex and its regulators* The interaction between Smc1, Smc3 and

Scc1/RAD21 creates an extended ring-like structure with a diameter of approximately 30-40nm. (Onn, Heidinger-Pauli et al. 2008). The cohesin core complex also includes a fourth subunit, Scc3/SA1, SA2, that interacts directly with Scc1 (Haering, Lowe et al. 2002; Michaelis, Ciosk et al. 1997). In most organisms, additional cohesin associated proteins have been identified. Pds5 transiently interacts with Scc1, but also interacts with other cohesin associated proteins, namely Rad61/WAPL, Eco1/ESCO1, ESCO2 and in vertebrates also the sororin protein (Nishiyama, Ladurner et al. 2010). All these proteins are weakly associated with the cohesin complex to regulate the interaction of cohesin with chromatin and control whether cohesin should be in a cohesive or non-cohesive state (Panizza, Tanaka et al. 2000). The regulation of cohesion will be further discussed in the section of sister chromatid cohesion establishment.

	<i>Saccharomyces cerevisiae</i>	<i>Homo sapiens</i>
<b>Cohesin core complex</b>	SMC proteins	Smc1 SMC1A
		Smc3 SMC3
	kleisin subunits	Scc1/Mcd1 RAD21
	kleisin binding proteins	Scc3 SA1/STAG1, SA2/STAG2
<b>Cohesin regulators</b>	kleisin binding proteins	Pds5 PDS5A, PDS5B
	Kollerin loading complex	Scc2 NIPBL
		Scc4 MAU2/hSCC4
	Cohesin acetyltransferases	Eco1/Ctf7 ESCO1, ESCO2
	Cohesin deacetylase	Hos1 HDAC8
	Pds5 binding proteins	Rad61/Wapl WAPL
	-	sororin

*Table1. Components of the mitotic cohesin complex and cohesin regulatory proteins in yeast and human*

## 3.2. Cohesin on chromatin

### 3.2.1 How cohesin holds sister chromatids together- the one ring model

Because the Smc1, Smc3 and Scc1/RAD21 subunits of the cohesin complex bind to each other in a way that creates an elongated ring-like structure, a model has been proposed that cohesin mediates cohesion by encircling the sister chromatid pairs as a ring (Haering, Lowe et al. 2002, Gruber, Haering et al. 2003, Haering, Farcas et al. 2008). This model for chromatin association has been referred to as the “one ring” model or “embrace” model. According to this model a single monomeric cohesin ring can embrace two 10nm DNA fibers topologically. However, other models have been proposed and even though there is very persuasive evidence for the ring model, excluding the alternative models today would potentially be jumping to conclusions prematurely (Huang, Milutinovich et al. 2005; Milutinovich, Unal et al. 2007; Diaz-Martinez, Gimenez-Abian et al. 2007; Guacci 2007)

### 3.2.2 Cohesin deposition

Regardless of the controversies surrounding the various cohesion models, it is well established that cohesin binds to chromatin. Cohesin deposition onto chromatin has

been shown to be regulated both spatially and temporally. In yeast, cohesin loading takes place in late G1 phase, while in vertebrates cohesin is loaded onto chromatin already in telophase, at the end of mitosis. Mechanistically, cohesin loading has been shown to depend on the loading complex Scc2-Scc4, also known as adherin (Furuya, Takahashi et al. 1998) or kollerin (Nasmyth 2011). The loading complex will from here on be referred to as kollerin. The kollerin complex is a heterodimer with two orthologs in all organisms analyzed. The Scc2/NIPBL subunit of kollerin is a large protein (>350 kDa human ortholog). Despite the lack of great sequence similarities between orthologs, the domain architecture is evolutionary conserved, where the N- terminal is believed to interact with Scc4/MAU2 and the C- terminal has been suggested to interact with cohesin (Oka, Suzuki et al. 2011; Braunholz, Hullings et al. 2012). Experiments in yeast indicate that kollerin is not required for the assembly of the cohesin complex, but only for cohesin loading (Ciosk, Shirayama et al. 2000). The requirement for kollerin in cohesin deposition is conserved throughout evolution, however the molecular mechanism behind cohesin loading onto chromatin remains poorly understood. One possible scenario for cohesin deposition by kollerin, is to simply tether a cohesin subunit to chromatin and thereby juxtapose cohesin assembly next to chromatin instead of occurring in solution. Furthermore, the mechanism behind how kollerin itself is recruited to DNA is not clear. Studies in different species propose that also kollerin association to DNA is regulated differently between organisms (Liu and Krantz 2008).

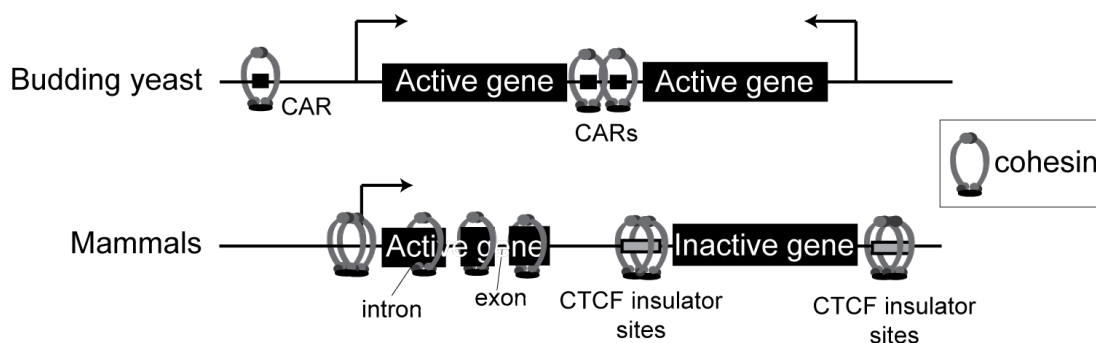
Given that cohesin is preassembled prior to chromatin loading and that cohesin is believed to embrace the DNA as a ring, the deposition of cohesin onto DNA requires a transient opening of the cohesin ring structure. It has been proposed that the hinge domains of Smc1 and Smc3 have to be separated from each other to form an “entry gate” for DNA (Gruber, Arumugam et al. 2006; Mishra, Hu et al. 2010; Nasmyth 2011; Mishra, Hu et al. 2010). Mechanistically, this has been suggested to rely on the ATPase activity of the head domains of Smc1 and Smc3, where ATP binding and hydrolysis potentially lead to a dramatic conformational change affecting also the hinge domain (Gruber, Haering et al. 2003; Arumugam, Gruber et al. 2003; Weitzer, Lehane et al. 2003).

### **3.2.3. Cohesin positioning**

Already in 1999, Nasmyth’s group observed that yeast Scc2 and cohesin subunits did not colocalize in chromosome spreads. Nevertheless, Scc2 was shown to be required for cohesin association with chromatin (Toth, Ciosk et al. 1999). With the development of new techniques such as Chromatid Immunoprecipitation (ChIP) in combination with -chip or sequencing (-seq), it became possible to globally map DNA-binding sites, precisely for your protein of interest. Using ChIP-chip in budding yeast, Nasmyth’s observation was confirmed, cohesin and kollerin are localized at different sites (with the exception of centromeres) (Lengronne, Katou et al. 2004; Glynn, Megee et al. 2004). This observation led to the proposal that cohesin is first loaded onto chromatin at the kollerin binding sites and subsequently translocated to its permanent sites. How cohesin is relocated from one site to the other is not clear, but mapping of cohesin binding in several organisms reveals that cohesin is positioned at specific sites and not distributed randomly, proposing that cohesin repositioning is an active process (Peters and Nishiyama 2012).

With the exception of the centromeric regions, the cohesin binding sites diverge greatly between organisms. In budding yeast cohesin is on average localized at 10-12kb intervals, each region spanning 0.8-1Kb (Mehta, Rizvi et al. 2012). These regions are known as cohesin-associated regions (CARs; Fig. 7). Budding yeast CARs lack a consensus sequence but are frequently found to be more AT- rich than average. Many of the budding yeast CAR sites are found in regions of convergent transcription and this finding led to the proposal that the pushing force of RNA polymerase II and the transcription machinery translocates cohesin to its permanent sites (Glynn, Megee et al. 2004; Lengronne, Katou et al. 2004).

In mammalian cells, another binding pattern of cohesin has been observed. In human cells, many sites are found in introns and directly upstream or downstream of genes, but also at intergenic regions (Fig. 7; Wendt 2008). Unlike in yeast, in mammalian cells no obvious enrichment between convergent genes is found (Parelho, Hadjur et al. 2008) (Wendt, Yoshida et al. 2008). ChIP-chip experiments in mammalian cells have identified a strong colocalization between cohesin and binding sites of the CTCF insulator (Parelho, Hadjur et al. 2008; Stedman, Kang et al. 2008; Wendt, Yoshida et al. 2008). RNA interference (RNAi) mediated knockdown of CTCF reduces the cohesin binding at CTCF-sites but without affecting the overall cohesin binding on chromatin, suggesting that CTCF is responsible for correct positioning of cohesin but not for the initial loading (Parelho, Hadjur et al. 2008; Stedman, Kang et al. 2008; Wendt, Yoshida et al. 2008). How cohesin moves from its loading sites to CTCF sites remains unknown, but unlike in yeast, there is no evidence that transcription alters the pattern of cohesin binding in mammalian cells.



**Figure 7. Schematic illustration showing relative positions of cohesin in yeast and mammals**

In conclusion, how cohesin is relocated from its loading positions to its more permanent sites remains largely unknown and studies suggest that it takes place differently in different organisms. Worth mentioning is an alternative explanation presented by the Nasmyth group. According to this model, instead of cohesin translocation on chromatin, cohesin is first loaded onto sites where kollerin binds but dissociates to be reloaded at its permanent positions (Hu, Itoh et al. 2011).

### 3.3. Cohesin and sister chromatid cohesion

The canonical role of the cohesin complex is to hold the two sister chromatids together from the time they are generated to the time of separation in mitosis (Fig. 8). Lack of sister chromatid cohesion leads to precocious sister chromatid separation, inevitably affecting the viability of the cell. It is well established that binding alone of cohesin to DNA is not sufficient to generate cohesion. Rather, chromatin-bound cohesin becomes cohesive in a separated and essential process. Sister chromatid cohesion is generated in close connection to DNA replication, which provides an elegant way to assure cohesion formed between sister chromatids and not between non-sister DNA molecules. Establishment of cohesion has been shown to depend on Eco1/ESCO1, ESCO2 an essential and evolutionary conserved acetyltransferase (Toth, Ciosk et al. 1999; Ben-Shahar, Heeger et al. 2008). In both yeast and mammals Eco1 and its orthologs acetylate the Smc3 subunit of the cohesin complex in S phase (Zhang, Shi et al. 2008). This modification has been shown to be essential for cell survival, but the mechanism by which this modification enables cohesin to become cohesive is not clear (Unal, Heidinger-Pauli et al. 2008; Zhang, Shi et al. 2008). The observation that cohesin has a very dynamic association with DNA in G1, whereas after DNA replication, the cohesin complex becomes more stably bound to DNA fits well with the notion that sister chromatid cohesion is generated in S phase (Gerlich, Koch et al. 2006). The DNA polymerase processivity factor PCNA has also been shown to be required for S phase cohesion, where it has been suggested to recruit Eco1 to chromatin (Moldovan, Pfander et al. 2006). Eco1 activity is normally constrained to S phase, since it is down regulated in a CDK1-dependent manner in G2 (Lyons and Morgan 2011).

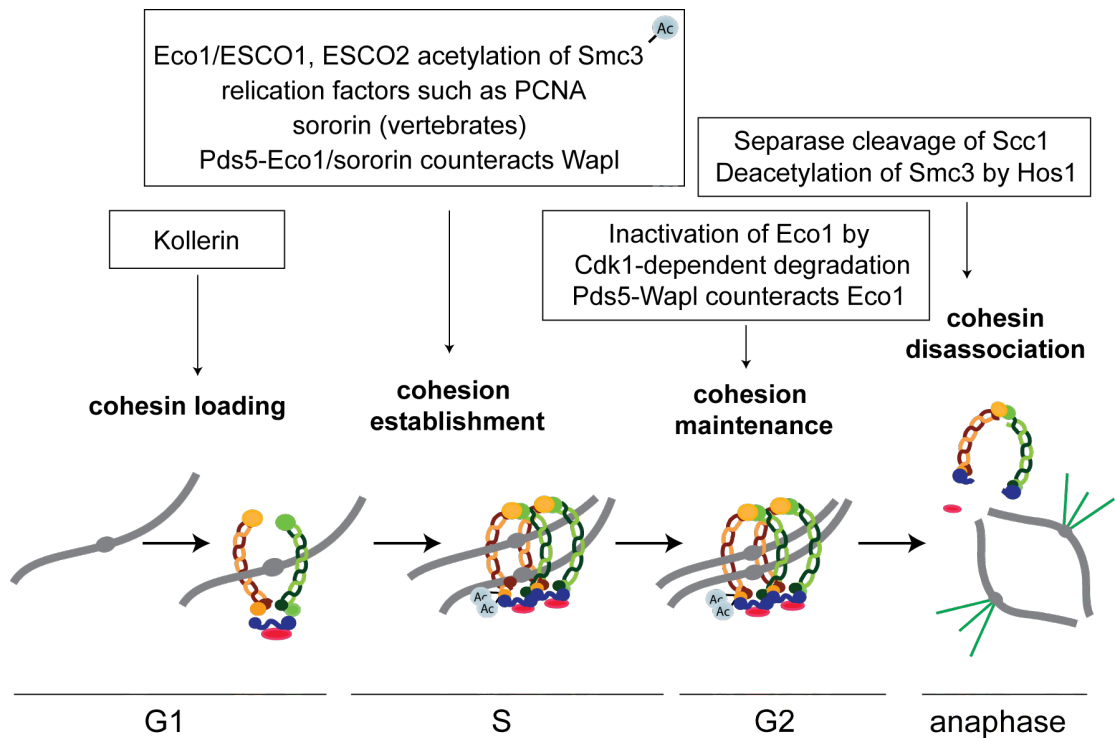


Figure 8. Schematic illustration of cohesin loading, establishment and removal in budding yeast

As mentioned in previous sections, several additional proteins have been identified that weakly associate with the cohesin complex to control whether cohesin is in a cohesive or non-cohesive state (Panizza, Tanaka et al. 2000). Pds5 is a large protein known to transiently interact with Scc1/RAD21 in both yeast and human (Panizza, Tanaka et al. 2000; Kulemzina, Schumacher et al. 2012). Pds5 was initially described as a cohesion maintenance factor, not required for cohesin deposition or for establishment of S phase cohesion, but solely for maintaining cohesion from S phase to mitosis (Panizza, Tanaka et al. 2000). Recent studies in budding yeast suggest that the importance of Pds5 goes beyond maintenance, by mediating the acetylation of Smc3 by Eco1 during S phase (Chan, Gligoris et al. 2013). Establishment of stable cohesion in S phase requires inactivation of the “anti-establishment” activity of Wapl, another factor known to weakly associate with cohesin. In budding yeast, Eco1 antagonizes Wapl as the Eco1-dependent acetylation of Smc3 displaces Wapl-Pds5 from chromatin (Rowland, Roig et al. 2009; Sutani, Kawaguchi et al. 2009). In vertebrates, WAPL is antagonized by sororin, an additional essential factor for creating stable cohesion, which is recruited by PDS5 following ESCO1 and ESCO2 acetylation of SMC3 (Rankin, Ayad et al. 2005; Nishiyama, Ladurner et al.). Pds5 can possibly be seen as a molecular bridge through which cohesin regulatory proteins interact with cohesin and control the “cohesiveness” of the cohesin complex. Because cohesin physically holds the two sister chromatids together the complex has to be removed in order for the sister chromatids to be separated in mitosis. The regulation of the cohesin removal from DNA is different in yeast and mammals. In budding yeast both arm and centromeric cohesin are removed by a regulated cleavage of the Scc1 subunit by the separase nuclease at the onset of anaphase (Uhlmann, Lottspeich et al. 1999). In vertebrates, cohesin is removed in two steps in which the separase independent “prophase pathway” removes the arm cohesin, and subsequently the separase enzyme removes the centromeric cohesin at the anaphase onset (Waizenegger, Hauf et al. 2000). This “prophase pathway” seems to depend on WAPL anti-establishment activity and inactivation of sororin (Nishiyama, Ladurner et al.; Gandhi, Gillespie et al. 2006; Kueng, Hegemann et al. 2006).

An additional crucial event in cohesin removal is deacetylation of the Smc3 subunit, performed by Hos1/HDAC8 which in budding yeast has been shown to depend on Scc1 cleavage by separase (Beckouet, Hu et al. 2010; Deardorff, Bando et al. 2012; Borges, Lehane et al. 2010; Xiong, Lu et al. 2010).

### **3.4. Cohesin and DNA damage response**

#### **3.4.1 Cohesin and DSB repair**

Years before Koshland and Hartwell monitored sister chromatid separation and speculated on potential protein (-s) serving the function of holding sister chromatids together it was observed that DNA repair efficiency increases dramatically when budding yeast cells go from G1 to G2 (Brunborg and Williamson 1978). This observation indicated that completion of DNA replication was an important determinant for efficient DNA repair. Following the discovery of the cohesin complex together with experiments indicating that both cohesin and cohesion were indispensable for DSB repair in postreplicative cells, the connection between cohesin and DNA repair became easy to conceive (Sjogren and Nasmyth 2001). As discussed in the previous sections, DSBs induced in S and G2 phases are preferentially repaired by HR using the

sister chromatid as a template for the repair, a mechanism that might be facilitated by cohesion between the chromatids. In addition to S phase cohesion, the cohesin complex has been shown to have other more direct roles in the DNA damage response and DSB repair.

### **3.4.2 Damage-induced sister chromatid cohesion**

In budding yeast, in response to a DSB induced in G2/M arrested cells, cohesion establishment is reactivated. This reestablishment of cohesion has been termed damage-induced (DI) cohesion and involves new kollerin-dependent loading of cohesin to the break site and new cohesion generation at the vicinity of the break and also on undamaged chromosomes (Strom, Lindroos et al. 2004; Unal, Arbel-Eden et al. 2004). DI cohesion has been shown to be regulated by the DNA damage response factors, Mec1, Tel1 and Mre11 as well as phosphorylation of the H2A. While cohesion generation in S phase is strongly connected to DNA replication, DI cohesion was shown to be independent of the same (Strom, Karlsson et al. 2007; Unal, Heidinger-Pauli et al. 2007). Overexpression of Eco1 bypasses the requirement for a DSB in G2, suggesting that Eco1 activity is the limiting factor in undamaged G2 cells (Unal, Heidinger-Pauli et al. 2007). The mechanism that makes chromatin-bound cohesin cohesive in response to a DSB involves Eco1 activity but seems to differ from S phase cohesion, where studies from Koshland's group suggest that following DSBs in postreplicative yeast cells Eco1 acetylates the Scc1 subunit of cohesin to establish DI cohesion, and that this Scc1 acetylation is triggered by phosphorylation of Scc1 by the checkpoint kinase Chk1 (Heidinger-Pauli, Unal et al. 2008, Heidinger-Pauli, Unal et al. 2009). As for S phase cohesion, formation of DI cohesion is counteracted by the "anti-establishment" of Wapl (Heidinger-Pauli, Unal et al. 2009).

It is attractive to think that DI cohesion would be required for DSB repair for the same reason as S phase cohesion, providing an excellent way to control the usage of the sister chromatid as a template for the repair by HR. However, analyzing additional factors in the regulation of DI cohesion reveals that this might be an oversimplification. Several factors were found to be required for full DI cohesion generation while they were dispensable for DSB repair (Sjogren and Strom 2010).

### **3.4.3 Cohesin and DNA damage checkpoint activation**

Several studies point towards a role of cohesin in DNA damage checkpoint activation. An intra-S phase checkpoint function was suggested, as the cohesin subunits Smc1 and Smc3 become phosphorylated by ATM in response to DNA damage, and this modification was shown to be important to properly block DNA replication (Luo, Li et al. 2008). Cohesin has also been proposed to have a role in G1 and the G2/M DNA damage checkpoint, where it was shown that RNAi mediated downregulation of RAD21 resulted in deficient recruitment of the DNA damage response factor 53BP1 to DSBs and a weaker activation of the checkpoint kinase Chk2 (Watrin and Peters 2009). Interestingly, the checkpoint function of cohesin was shown to be independent of cohesion, as cells depleted of the establishment and maintenance factor sororin did not impair G2/M or intra-S checkpoints, whereas cohesin depletion did (Watrin and Peters 2009).

## 4. DNA POLYMERASE ETA ( $\eta$ )

### 4.1 Pol $\eta$ in translesion DNA synthesis

DNA polymerase  $\eta$  (Pol $\eta$ ) belongs to a class of DNA polymerases termed Translesion DNA Synthesis (TLS) polymerases. TLS is the process by which a DNA lesion is bypassed by the incorporation of nucleotide(s) opposite the lesion (Waters, Minesinger et al. 2009). Because of the structural constraints DNA lesions can impose, many DNA lesions cannot be used as a template by the canonical replicative polymerases. Instead, replication over damaged DNA is often performed by the TLS polymerases, which have a more open configuration of their active sites, enable them to tolerate damaged DNA and more efficiently synthesize past the lesion (Prakash, Johnson et al. 2005). TLS polymerases are found in all three domains of life and most of them belong to the Y-family of polymerases, which in budding yeast includes Pol $\eta$  and Rev1 (Waters, Minesinger et al. 2009). In addition, budding yeast also express one non Y-family polymerase named DNA Pol $\zeta$  (Rev3/Rev7), which also functions as a TLS (Lawrence 2004).

In budding yeast, Pol $\eta$  is encoded by the *RAD30* gene and is the preferred polymerase for reading through ultraviolet (UV) lesions (McDonald, Levine et al. 1997; Johnson, Prakash et al. 1999). Inactivation of Pol $\eta$  in budding yeast results in hypersensitivity to UV light and leads to an increase in UV-induced mutation frequencies (McDonald, Levine et al. 1997; Johnson, Prakash et al. 1999). In humans, inactivation of Pol $\eta$ , leads to a variant form of the cancer prone syndrome xeroderma pigmentosum (XP-V) (McDonald, Levine et al. 1997; Masutani, Kusumoto et al. 1999). Cells derived from individuals with XP-V display deficiency in replication over UV-damaged DNA and show increased mutation frequency in response to UV irradiation (Cleaver 1981; Lehmann, Kirk-Bell et al. 1975; Wang, Maher et al. 1993).

One of the key steps in TLS is the polymerase switch, in which the replicative polymerase is switched to one of the TLS polymerases. This has been shown to depend on a monoubiquitination of PCNA at K164, with which Pol $\eta$  is known to interact (Kannouche, Wing et al. 2004). Budding yeast Pol $\eta$  contains a ubiquitin-binding zinc-domain (UBZ) and a PCNA-interacting peptide (PIP) both known to be required for the interaction with PCNA (Hoegge, Pfander et al. 2002; Bienko, Green et al. 2005; Parker, Bielen et al. 2007).

TLS polymerases have also been assigned with a gap-filling function outside of S phase and independent of the replication fork. When the replication machinery re-starts downstream of a blocked DNA lesion it results in ssDNA gaps, and these gaps are presumably sealed by TLS polymerases (Sabbioneda, Bortolomai et al. 2007; Waters and Walker 2006). Furthermore, Pol $\eta$  has been suggested to have an important function in HR, specifically in the extension of the D-loop structure (Kawamoto, Araki et al. 2005; McIlwraith, Vaisman et al. 2005).



## 4.2 Implications for Pol $\eta$ in cohesion establishment

One of the indications that Pol $\eta$  could be important for DI cohesion came from the finding that the fission yeast *Schizosaccharomyces pombe* Eso1 protein contains two separable protein domains, where the amino-terminal end is highly homologous to budding yeast and human Pol $\eta$  and the carboxyl-terminal end is highly homologous to the budding yeast Eco1 protein (Fig. 9; Tanaka, Yonekawa et al. 2000). Deletion analyses have indicated that the two protein domains are independent and retain their respective functions in damage bypass and sister chromatid cohesion respectively (Tanaka, Yonekawa et al. 2000; Madril, Johnson et al. 2001). The contribution of the Pol $\eta$  domain for S phase cohesion has indeed been tested and shows to be minimal and the fact that *RAD30* is not an essential gene gives a further indication that Pol $\eta$  is dispensable for S phase sister chromatid cohesion. However, since Eco1 also is the major regulator of DI cohesion, it could potentially assign Pol $\eta$  with a function specifically in DI cohesion.

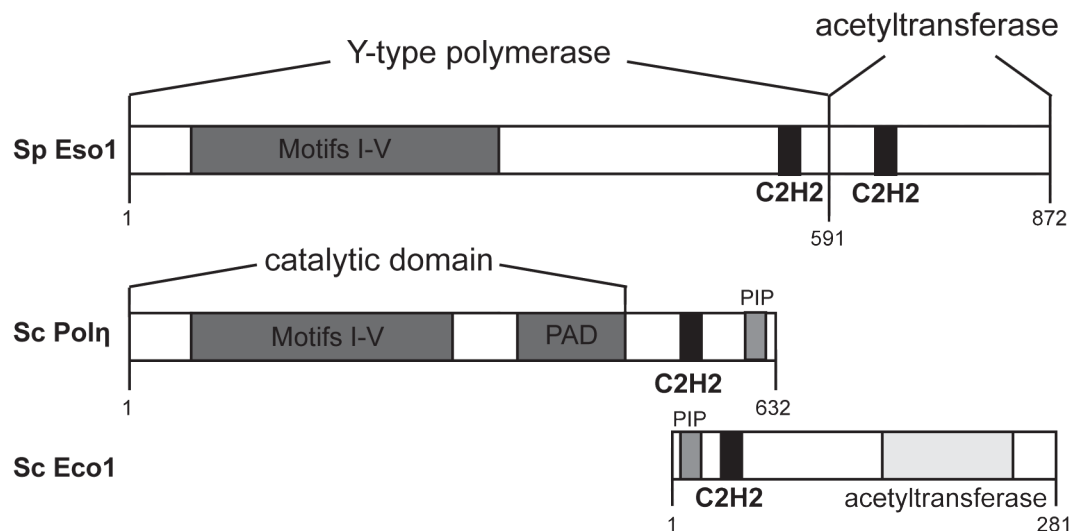


Figure 9. Alignment of fission yeast Eso1 with budding yeast Pol $\eta$  and Eco1

Another indication that Pol $\eta$  could function in DI cohesion is the connection to PCNA. As mentioned above, the interaction between Pol $\eta$  and PCNA is essential for Pol $\eta$  function in TLS and likewise, Eco1 has been shown to interact with PCNA during S phase cohesion establishment (Moldovan, Pfander et al. 2006).

## 5. WHEN COHESIN FUNCTIONALITY FAILS

### 5.1 Cohesinopathies

Cohesinopathies is a term used to describe human disorders associated with mutations in cohesin subunits and cohesin-associated proteins. Cohesinopathies includes Cornelia de Lange syndrome (CdLS; OMIM #122470), Roberts syndrome (RBS; OMIM #268300) and Warsaw breakage syndrome (OMIM #613398). Here CdLS and RBS will be discussed further.

### 5.1.1 Cornelia de Lange Syndrome

Cornelia de Lange syndrome (CdLS), also known as Brachmann-de Lange syndrome is a rare developmental disorder. The syndrome was first described by Vrolik and Brachmann but the diagnostic criteria of this disorder was later proposed by De Lange (Brachmann 1916; Oostra, Baljet et al. 1994; de Lange 1933). The prevalence of CdLS has been estimated between 1: 10 000 and 1: 50 000 births (Opitz 1985; Barisic, Tokic et al. 2008), where most cases are sporadic. The characteristic facial features are the most consistent and recognizable in CdLS. Most individuals show low anterior hairline, arched eyebrows, eyebrows growing across the base of the nose (synophrys), unusually long eyelashes and a thin upper lip with down-turned corners (Jackson, Kline et al. 1993). In addition, CdLS individuals often suffer from pre- and postnatal growth retardation, mental retardation and upper limb anomalies (Jackson, Kline et al. 1993). Multiple internal organs are often affected in CdLS, such as renal malformations and congenital heart defects. Gastroesophageal reflux disease is also common among these individuals (Liu and Krantz 2009).

A screen for candidate CdLS genes demonstrated that many affected individuals carry mutations in the *NIPBL* gene (Krantz, McCallum et al. 2004). About 60% of the individuals diagnosed with CdLS have a heterozygous mutation in *NIPBL*. Genotype-phenotype correlations suggest that haploinsufficient *NIPBL* mutations (protein-truncating mutations) usually result in a more severe phenotype while missense mutations cause a milder phenotype, however this is not always true (Gillis, McCallum et al. 2004). Mutations in the cohesin core components, *SMC1A*, *SMC3* and *RAD21* and cohesin-associated genes *PDS5b* and *HDAC8* have also been linked to CdLS (Deardorff, Kaur et al. 2007; Deardorff, Bando et al. 2012; Deardorff, Wilde et al. 2012; Zhang, Chang et al. 2009).

The canonical function of cohesin in sister chromatid cohesion and chromosome segregation is most likely not the underlying molecular mechanism by which cohesin influences developmental control. The more recently discovered function of cohesin in regulating gene expression by enabling interactions between long-range regulatory elements is the more likely cause of the developmental defects seen in CdLS (Dorsett 2009; Dorsett 2011; Liu and Krantz 2008). *NIPBL* mutations found in CdLS individuals have been shown to affect chromatin cohesin loading, suggesting that CdLS may be caused by alterations in cohesin chromatin binding dynamics leading to transcriptional dysregulation (Liu, Zhang et al. 2009).

### 5.1.2 Roberts syndrome

Roberts Syndrome (RBS) is an autosomal recessive disorder caused by mutations in the human *ESCO2* gene (Vega, Waisfisz et al. 2005). The clinical features of RBS are distinct from CdLS, with some overlap. Some of the specific characteristics for RBS are; symmetrical limb reductions often affecting all four limbs, abnormally increased distance between the eyes (orbital hypertelorism) and cleft palate. As with CdLS, RBS symptoms include pre- and postnatal growth deficiency and mental retardation (Dorsett 2007; Liu and Krantz 2009). The mutations in *ESCO2*, identified in individuals diagnosed with RBS, result in disruption of the acetyltransferase domain, suggesting that the acetyltransferase activity of *ESCO2* is important for the embryonic development (Gordillo, Vega et al. 2008).

## 5.2 Cancer development

There is increasing evidence linking dysfunctional cohesin to the development of human cancers. *NIPBL*, *SMC1A*, and *SMC3* have been found mutated in colorectal cancers and knockdown of *SMC1A* and *SMC3* resulted in disruption of the expression of two genes involved in suppressing chromosome instabilities (CIN; Barber, McManus et al. 2008). These results suggested that mutations in the cohesin subunits or associated proteins could lead to CIN in colorectal cancers (Barber, McManus et al. 2008). However, *SMC1*, *SMC3*, *RAD21* and *SA2* have also been found mutated in acute myeloid leukemia, a cancer type not characterized by aneuploidy and CIN (Welch, Ley et al. 2012). Individuals with CdLS do not display an increased risk of developing cancer, neither does the *NIPBL* heterozygous mouse. However, mice heterozygous for *SA1* show increased aneuploidy and tumorigenesis (Remeseiro, Cuadrado et al. 2012). One of the future challenges is to understand how dysfunctional cohesin contribute to cancer development. Given that cohesin is a multifunctional complex, this question will most likely be provided with several answers.

## 2. COMMENTS ON METHODOLOGY

### Choosing appropriate models

Model organisms are often chosen on the basis of size, generation time, maintenance costs and the ability to be genetically manipulated. However, the choice also depends on the questions addressed and the specific experiments involved.

#### 1. Yeast models

*Saccharomyces (S.) cerevisiae*, (budding yeast) has a haploid genome composed of a total of 2200 kilobases (kb), organized on 16 chromosomes. Budding yeast has approximately 6000 genes, of which we know the function of about 5000 and is the organism we today know most details of, and consequently has a well-defined genetic system. Yeast is in many aspects an ideal model organism. It is a unicellular eukaryote, has a short generation time and it is relatively easy and cheap to house and maintain. In addition, budding yeast has a highly efficient DNA recombination system that enables *in vivo* recombination of transformed linear DNA with homologous genomic DNA. This makes gene deletions, gene modifications and epitope tagging a relatively straightforward process. Yeast is non-pathogenic so no precautions are needed when handling, and moreover working with yeast brings no ethical concerns. Despite the fact that yeast is a unicellular organism and that humans are genetically more complex than yeast, we share many fundamental molecular mechanisms and data obtained from yeast can in many cases be transferred and applied to human cells.

#### 2. Human cell culture models

Cell systems are amenable to experimentally address questions that may not be feasible in whole animals and include cell lines and primary cell cultures. Commercially available cell lines proliferate well and can easily be propagated in culture. An advantage of cell lines is that they are considered relatively homogenous biological

models. However, many cell lines are derived from tumors and it is questionable how well they resemble the tissue from where they originate. Primary cell cultures are cells isolated directly from an organism and placed in an environment where they can grow. Primary cells could be considered as physiologically closer to the tissue from where it originated but with the drawback that primary cell cultures contain a very heterogeneous population of cells. Another limitation is that primary cells have a limited life span, entering senescence after a certain number of cell divisions (Hayflick and Moorhead 1961; Hayflick 1965). RNAi is a relatively new but today very frequently used technique for analyzing genes and their function. The method allows for post-transcriptional silencing of a specific gene without modifying the genome (Fire, Xu et al. 1998). RNAi is commonly used for analyzing gene functions in cell culture systems.

Comparing the effects of various treatments on cells from healthy controls and patient-derived cells is a relatively common approach to investigate the functions of disease-causing genes. However, individual variation within a control group and patient group, can mask putative phenotypes. Therefore, the number of individuals to include in a comparative research study is an important consideration. Nevertheless, the availability of patient material is often a limiting factor in these studies. In addition, working with patient material always includes applying for ethical permissions.

### 3. AIMS

The overall aim of this thesis has been to elucidate how regulation of cohesin affects cells ability to functionally respond to and repair DSBs. For this we have used human cell cultures (paper I) and the budding yeast model system (paper II and III). Specific questions addressed in the papers are:

- (I) Do cells derived from individuals diagnosed with CdLS display an increased sensitivity to the induction of DSBs and how are DSBs repaired in these cells? CdLS is caused by mutations in the NIPBL gene, required for cohesin loading to chromatin. Using NIPBL deficient cells derived from CdLS individuals we investigated how dysfunctional NIPBL affects the ability of the cells to handle DSBs.
- (II) How does the absence of DNA polymerase  $\eta$  affect the ability of budding yeast cells to generate DI cohesion and repair DSBs induced post replication? In this paper we also addressed the question whether DI cohesion at the vicinity of a DSB and on undamaged chromosomes is regulated differently.
- (III) Is DNA polymerase  $\eta$  regulated by the acetyltransferase Eco1 for DI cohesion? This is a study where we further investigated the role of DNA polymerase  $\eta$  in DI cohesion described in paper II. Here we aimed at revealing the molecular mechanism by which DNA polymerase  $\eta$  is involved in DI cohesion.

All human studies were performed according to the Declaration of Helsinki and received appropriate ethical approvals.

## 4. RESULTS AND DISCUSSION

### 4.1 PAPER I: A REGULATORY ROLE FOR THE COHESIN LOADER NIPBL IN NON HOMOLOGOUS END JOINING DURING IMMUNOGLOBULIN CLASS SWITCH RECOMBINATION

The aim of this paper was to address the role of NIPBL in repairing DSBs. In doing so, we used B-lymphocytes and primary fibroblasts isolated from individuals diagnosed with CdLS. Cells from healthy individuals, ATM deficient, Cernunnos deficient and RBS individual were used as controls. We also mimicked the CdLS genotype by knocking down NIPBL using siRNA in human cell cultures. Moreover, we analyzed *in vivo* recombined switch junctions in B-cells from CdLS individuals.

#### Increased DNA damage sensitivity in CdLS cell lines

To investigate whether CdLS cells demonstrate increased sensitivity to DSBs we exposed CdLS cells and controls to  $\gamma$ -IR, Mitomycin-C or etoposide. A significantly increased DNA damage sensitivity was observed in CdLS cells compared to healthy control cells, especially after exposure to  $\gamma$ -IR. To directly test whether dysfunctional NIPBL was the underlying cause for increased DNA damage sensitivity in CdLS cells we performed a NIPBL knockdown experiment using siRNA targeted to NIPBL, and analyzed the sensitivity to  $\gamma$ -IR. RNAi mediated downregulation of NIPBL protein levels phenocopied the CdLS cells and caused a significant increase in sensitivity to  $\gamma$ -IR suggesting that functional NIPBL is required for correct repair of  $\gamma$ -IR induced DSBs.

#### DSB repair via NHEJ is impaired in NIPBL deficient cells

When analyzing the cell cycle profiles of these cells we observed that the majority of the cells were in G1 phase at the time of treatment. To us, this suggested that NHEJ could be defective in these cells. Loss of functional C-NHEJ factors (e.g. Ku80 and XRCC4), have been shown to cause increased frequency of microhomology-mediated end joining, characteristic of the A-EJ repair mechanism (Kabotyanski, Gomelsky et al. 1998). Consequently, we aimed at investigating how DSB end joining is performed in NIPBL deficient CdLS cells. In CSR, DSBs are purposefully induced to create genetic rearrangement enabling immunoglobulin (Ig) diversity. CSR is initiated by activation-induced cytidine deaminase (AID), resulting in DSBs in the donor and acceptor switch (S) regions (Muramatsu, Kinoshita et al. 2000). The intervening sequence is removed and the S regions are joined. NHEJ is considered to be the primary mechanism used for DSB repair in CSR, as AID-dependent DNA breaks are introduced and repaired mainly in the G1 phase of the cell cycle (Schrader (Schrader, Guikema et al. 2007). Analyzing *in vivo* switch recombination junctions in CdLS individuals is therefore an ideal approach to study NIPBL contribution to the mode of end joining. By analyzing S $\mu$ -S $\alpha$  junctions from CdLS individuals with known NIPBL mutations, we found a significantly reduced proportion of S $\mu$ -S $\alpha$  junctions in NIPBL deficient B cells with

“direct end joining” (i.e. no microhomology) compared to healthy controls (Fig. 10). Conversely, a significantly increased proportion of the junctions from NIPBL deficient cells displayed microhomologies of varied length. A similar pattern was observed in CdLS individuals with *SMC1A* mutations, but with a less pronounced shift towards microhomology usage. This pattern largely resembles switch regions analyzed in cells with a known dysfunctional C-NHEJ pathway, suggesting that NIPBL serves a function in regulating C-NHEJ. To further analyze the end joining defect seen in CSR we performed an *in vitro* plasmid-based end joining assay, in both NIPBL deficient CdLS cells and NIPBL knockdown cells. In line with the data from the *in vivo* switch recombination junctions we found that NIPBL deficient cells display a shift towards microhomology usage in end joining repair.

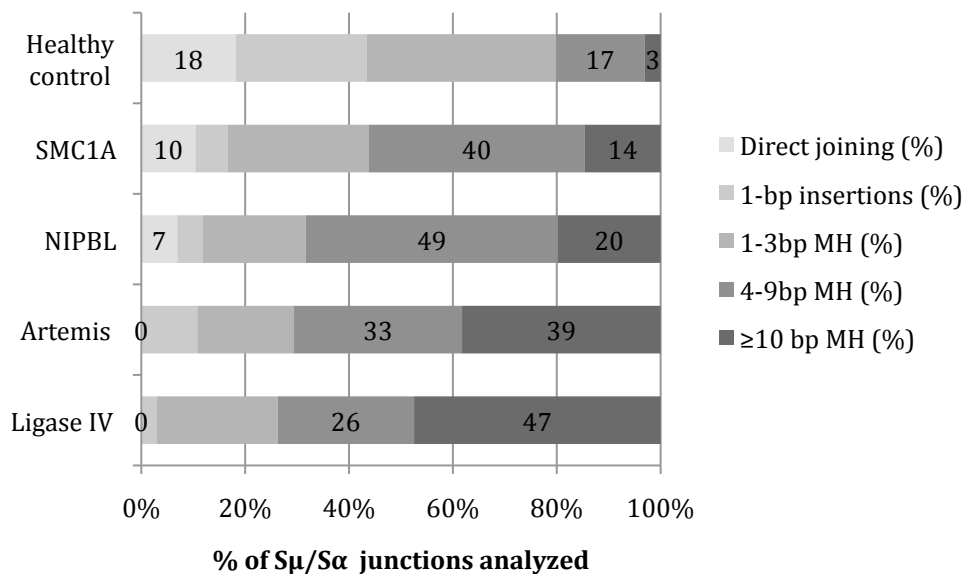


Figure 10.  $\Sigma\mu$ - $\Sigma\alpha$  junctions analyzed for microhomology (MH) usage in patient groups and control.

### Reduced recruitment of 53BP1 to $\gamma$ -IR induced DSBs in NIPBL deficient CdLS cells

To date, no cohesin-independent functions of NIPBL have been described. This suggests that the involvement of NIPBL in C-NHEJ is likely through the cohesin complex. In addition, it has been shown that CdLS cells have reduced levels of chromatin-bound cohesin (Liu, Zhang et al. 2009). Mutations in true C-NHEJ repair factors cause, in humans, pronounced immune-deficiency. Since this is not observed in CdLS individuals we believe that it is likely that NIPBL/cohesin is not direct components of the C-NHEJ repair pathway but rather a factor with a role in regulating the choice of repair pathway. It was previously shown that RNAi mediated downregulation of RAD21 results in deficient recruitment of the DNA damage response factor 53BP1 to DSBs (Watrin and Peters 2009). In addition, loss of 53BP1 has been shown to result in an increased resection of DNA ends induced in the Ig S regions, resulting in an increased frequency of microhomology-mediated end joining (Bothmer, Robbiani et al. 2010). To test whether deficient recruitment of 53BP1 could be the underlying mechanism by which CdLS cells display an increased sensitivity to DNA damage, we scored 53BP1 foci formation at selected time points following  $\gamma$ -IR

in NIPBL deficient CdLS and control cells. We found that at an early time point following DSB induction, the numbers of foci detected in CdLS cells were significantly reduced, however, at a later time point no difference was observed. This suggests that functional NIPBL is required for a proper recruitment of DNA damage response factors such as 53BP1 to DSBs in order to steer the repair towards the C-NHEJ pathway. The increased sensitivity to  $\gamma$ -IR observed in CdLS cells could potentially be explained by an increased usage of the more error-prone A-EJ repair mechanism, leading to large deletions and translocations incompatible with viability. Numerous chromosomal rearrangements have been reported in individuals with CdLS (DeScipio, Kaur et al. 2005), suggesting that impaired regulation of C-NHEJ could possibly contribute to the phenotypes seen in CdLS syndrome. Taken together, the data presented in this paper illuminates cohesin as a multifunctional complex.

### **Conclusions from paper I**

- \* Cells derived from individuals diagnosed with CdLS display an increased sensitivity to  $\gamma$ -IR.
- \* Abnormal switch junctions in CdLS patients' B cells that resemble those found in cells with a known dysfunctional C-NHEJ pathway. This abnormal pattern is characterized by an increased usage of microhomologies.
- \* NIPBL deficient cells show a significantly increased usage of microhomologies in a plasmid-based end joining assay.
- \* Reduced recruitment of 53BP1 to DSBs are found in NIPBL deficient CdLS cells following  $\gamma$ -IR.

## **4.2 PAPER II: IMPORTANCE OF POL $\eta$ FOR DAMAGE INDUCED COHESION REVEALS DIFFERENTIAL REGULATION OF COHESION ESTABLISHMENT AT THE BREAK SITE AND GENOME-WIDE**

The aim of this paper was to address the role of Pol $\eta$  in DI cohesion. For this we use budding yeast as a model organism. Sister chromatid separation was scored using the Tet-repressor–GFP/Tet-operators (Tet-R-GFP/Tet-O) system. This system utilizes the insertion of an array of Tet-operators on chromosome V, to which the endogenously expressed GFP-tagged Tet-repressor binds. This resulted in one green fluorescent spot in cells where the sisters were cohered and two spots where they were separated (Strom, Lindroos et al. 2004). DSB was predominantly induced using the galactose-inducible, site specific, HO-endonuclease, generating one DSB at the *MAT* locus on chromosome III. Consequently, the DSB was induced on chromosome III while sister chromosome separation was monitored on chromosome V. This means that any DSB-dependent cohesion that is observed must occur genome-wide.



### **Pol $\eta$ is required for formation of DI cohesion in response to $\gamma$ -IR as well as to induction of a single DSB**

Using two separate experimental systems, namely the Scc1 noncleavable (Scc1<sup>NC</sup>) and the Smc1 temperature sensitive system (smc1<sup>ts</sup>/Smc1<sup>WT</sup>), we investigated the importance of Pol $\eta$  for DI cohesion genome-wide. Our data clearly displayed that Pol $\eta$  is required for postreplicative DI cohesion genome-wide.

### **Genome-wide and DSB proximal DI cohesion are regulated differently**

It is easy to conceive that DI cohesion would be required for DSB repair in postreplicative cells. Therefore we wanted to test whether Pol $\eta$  deficient cells were dysfunctional in DSB repair. Surprisingly, loss of Pol $\eta$  did not affect the cells ability to repair DSBs induced in G2/M. We could also conclude that DSBs induced in G2/M arrested cells were repaired by HR. To us, this was at the time an unexpected finding since the lack of cohesion between the sister chromatids would impede HR. However, so far we had only analyzed genome-wide DI cohesion (i.e. on undamaged chromosomes). This result inspired us to investigate DI cohesion at the vicinity of the break. We inserted the HO recognition sites on chromosome V (4kb from the Tet-array) while removing the chromosome III recognition site. Interestingly, when monitoring sister chromatid separation at the break site in Pol $\eta$  deficient cells we found DI cohesion to be functional. In line with this, we also demonstrated that cohesin binding at the area surrounding DSB is normal in Pol $\eta$  deficient cells. This suggests that DI cohesion is regulated differently in the vicinity of the break and genome-wide and that Pol $\eta$  is only required for the latter.

### **Novel function of Pol $\eta$ in genome-wide DI cohesin**

To elucidate the potential mechanism for the involvement of Pol $\eta$  in genome-wide DI cohesion, we set out to test whether the action of Pol $\eta$  was through DNA damage bypass. TLS polymerase-dead Pol $\eta$  mutants and Pol $\eta$  mutants in which the interaction with PCNA was abolished were tested for their ability to generate genome-wide DI cohesion. With the exception of one of the tested polymerase-dead mutants (*rad30-D155A*), none of these mutants displayed DI cohesion defects, suggesting that the mechanism by which Pol $\eta$  functions in DI cohesion is different from its canonical damage bypass activity in TLS.

### **Implications for a functional connection between Pol $\eta$ and Eco1**

It has previously been shown that the Eco1 acetyltransferase activity is the limiting factor for DI cohesion (Strom and Sjogren 2007; Unal, Heidinger-Pauli et al. 2007; Lyons and Morgan 2011). Overexpression of either Eco1 or an acetylmimic version of the Eco1 target Scc1, bypasses the requirement for a DSB break to induce DI cohesion in postreplicative cells. Since the loading and positioning of cohesin are normal in Pol $\eta$  deficient cells we speculated that Pol $\eta$  defective cells are dysfunctional at the level of cohesion establishment. Eco1, being the key cohesion establishment factor, could potentially be dysfunctional in Pol $\eta$  deficient cells. To test the potential Pol $\eta$ - Eco1 relationship we analyzed whether; (i) overexpression of Eco1 or acetylmimic version of Scc1 rescued a DI cohesion deficiency caused by lack of Pol $\eta$  (ii) absence of Pol $\eta$  affected the damage-induced stability of Eco1 (iii) the absence of Pol $\eta$  influenced Eco1 acetyltransferase activity. Our data suggests that Pol $\eta$  is dispensable for Eco1

acetyltransferase activity (as measured by Smc3 acetylation) and for Eco1 protein stability following DSBs. However, we found that overexpression of both Eco1 and an acetylmimic version of Scc1 overcame the need for Pol $\eta$  in genome-wide DI cohesion. This result can be interpreted in two ways, either Pol $\eta$  functions in the same pathway as Eco1- Scc1 in genome-wide DI cohesion with Pol $\eta$  acting upstream of Eco1, or Pol $\eta$  functions in a parallel pathway of Eco1- Scc1 that becomes secondary when the Eco1- Scc1 pathway is over activated.

To address the specific relevance of genome-wide cohesion in contrast to DSB-proximal cohesion, we studied the survival capacity of Pol $\eta$  deficient cells following multiple rounds of DSB induction. In a situation with repeated DSB inductions, Pol $\eta$  deficient cells displayed a significantly reduced survival capacity compared to wild type cells. This indicates that genome wide DI cohesion is important for maintaining genome stability. We suggest that genome-wide DI cohesion could be important for correct chromosome segregation following release from a G2/M arrest.

Future studies addressing how Pol $\eta$  functions in genome-wide cohesion will advance our understanding of the relevance for genome-wide DI cohesion.

### **Conclusions from paper II**

- \* Pol $\eta$  is required for formation of cohesion in response to a DSB in G2, but not for cohesin loading or HR- mediated DSB repair.
- \* Pol $\eta$  is required for genome-wide DI cohesion specifically but dispensable for establishment of DSB-proximal DI cohesion.
- \* The mechanism by which Pol $\eta$  functions in genome-wide DI cohesion is not dependent on its polymerase activity or interaction with PCNA.
- \* DI cohesion deficiency caused by lack of Pol $\eta$  is rescued by overexpression of Eco1 and by overexpression of an acetylmimic version of Scc1.
- \* Genome-wide DI cohesion is crucial for cell survival following repeated DSB inductions. We provide data suggesting that genome-wide DI cohesion could be important for correct chromosome segregation following release from a G2/M arrest.

## **4.3 PAPER III: ACETYLATION OF DNA POLYMERASE $\eta$ BY ECO1 REGULATES ITS FUNCTION IN GENOME-WIDE DAMAGE-INDUCED COHESION**

This manuscript is a direct continuation of paper II. Here we further investigated the relationship between Pol $\eta$  and Eco1 in DI cohesion. Although this story is not completely finished, it provides interesting results giving the basis for tempting speculation regarding the regulation of Pol $\eta$  in genome-wide DI cohesion. Here we

present data suggesting that Pol $\eta$  is regulated by Eco1 activity in genome-wide DI cohesion.

### **Pol $\eta$ is acetylated by Eco1 *in vitro***

We imagined that if Pol $\eta$  and Eco1 interact following DNA damage, then Pol $\eta$  is expected to be a substrate of Eco1. We set out to test this *in vitro*. We found that Pol $\eta$  is indeed acetylated by Eco1 in an *in vitro* Eco1 acetylation assay. To identify lysine residues acetylated by Eco1 in Pol $\eta$ , the *in vitro* acetylation assay was repeated and the Pol $\eta$  protein was isolated and subjected to nano-liquid chromatography- tandem mass spectrometry (nLC-MS/MS). We identified several lysine residues that were acetylated in an Eco1-dependent manner.

### **Selecting candidate Eco1 substrates in Pol $\eta$**

To narrow down the potential, functionally important Eco1 substrates in Pol $\eta$  we turned to the literature. The protein interactions with Pol $\eta$  characterized so far, occur at the flexible C-terminal domain of Pol $\eta$ . We imagined that potential acetylations important for Pol $\eta$ 's role in DI cohesion would be expected to reside within the C-terminal region as well (Bienko, Green et al. 2005; Acharya, Haracska et al. 2007). In addition, analyzing the surrounding residues of the known Eco1 targets Scc1-K84 and Scc1-K210 and comparing them with the *in vitro* identified Eco1-dependent acetylated lysine residues in Pol $\eta$ 's C-terminus we found that K546 perfectly resembled an Eco1 substrate. We therefore selected K546 from the list as potentially the most likely target of Eco1 acetyltransferase activity. In addition, despite not being identified as an Eco1 substrate *in vitro*, we also selected K549 based on its attractive position two residues upstream of K546.

### **Pol $\eta$ K546 and K549 are required for establishment of damage-induced cohesion genome-wide**

To address the physiological importance of lysine K546 and K549, we created mutants of Pol $\eta$  in which these lysines were substituted with the nonacetyltable residue arginine (*pol $\eta$ -K-R*). To investigate the ability of the *pol $\eta$ -K-R* mutants to generate genome-wide DI cohesion we used the previously mentioned Scc1<sup>NC</sup> experimental system. Interestingly, both *pol $\eta$ -K546R* and *pol $\eta$ -K549R* mutants displayed a DI cohesion deficiency to a similar extent as Pol $\eta$  depleted cells. This suggests that the acetylation of Pol $\eta$  by Eco1 at K546 could be required for Pol $\eta$ 's role in DI cohesion. So far, we have no indication of K549 being a direct target of Eco1 acetyltransferase activity. Thus, K549 could either be a substrate for a different acetyltransferase, subjected to some other type of posttranslational modification in a DNA damage-specific manner or important for Eco1 recognition of K546. However, the direct function of K549 needs further investigation. Interestingly, neither the *pol $\eta$ -K546R* nor the *pol $\eta$ -K549R* mutant showed an increased sensitivity to UV irradiation, further supporting our previous notion that the function of Pol $\eta$  in DI cohesion is separate from its function in TLS. Thus far, the lack of *in vivo* data supporting acetylation on K546 is a limitation of this story. Hence, confirming Pol $\eta$  acetylation *in vivo* is one of the primary goals for the future.

So what is the function of Pol $\eta$  in genome-wide DI cohesion? Although this is an ongoing study it begins to shed light on the role of Pol $\eta$  in genome-wide DI cohesion.

Future studies with the DI cohesion mutants of Pol $\eta$  will develop our understanding of the molecular mechanism behind its action. Interestingly, the position of K546 and K549 is just at the border of the highly conserved UBZ domain. We previously showed that D570, needed for interaction with monoubiquitinated PCNA, is dispensable for Pol $\eta$  function in DI cohesion. However the importance of the C2H2 zinc-binding motif of the UBZ has not yet been analyzed in DI cohesion.

### Conclusions from paper III

\* Pol $\eta$  is acetylated by Eco1 *in vitro*.

\* Pol $\eta$  K546 and K549 are required for establishment of damage-induced cohesion, potentially through Eco1-dependent acetylation of K546.

## 5. PERSPECTIVES AND CONCLUDING REMARKS

Historically, cohesin has mainly been studied for its role in chromosome segregation. However, during the last decade cohesin has been assigned with additional roles in regulation of gene transcription, DNA repair and replication as well as recombination. The multiple functions of cohesin position this complex at the center stage of genomic integrity. Here we have investigated the role of cohesin and its loader NIPBL, in the yeast and human DNA damage response.

In **paper I** we show that B-cells derived from individuals with Cornelia de Lange Syndrome, a syndrome caused by heterozygous loss-of-function mutations in the gene encoding the cohesin loading protein NIPBL, display increased usage of an microhomology-based, alternative end joining mechanism during CSR. A-EJ has in the past been considered a “backup” pathway when C-NHEJ is failing, however more recent data suggest that A-EJ is a robust pathway and that A-EJ may compete with C-NHEJ also when C-NHEJ is functional (Yan, Boboila et al. 2007; Pan-Hammarstrom, Jones et al. 2005). In line with our findings, dysfunctional regulation of repair pathway choice and increased usage of A-EJ is often correlated with increased sensitivity to ionizing radiation (Yan, Boboila et al. 2007; Schar, Fasi et al. 2004). An important question is to what extent A-EJ contributes to the repair of exogenously induced DSBs? It has so far been technically challenging to study the role of A-EJ in repairing exogenously induced DSB. Thus, most A-EJ studies are based on end joining events in CSR and *in vitro* plasmid-based assays. It is possible that A-EJ may compete better with C-NHEJ for CSR events involving switch regions that contains a relatively high frequency of short repetitive sequences, thereby providing more optimal microhomology substrates for A-EJ. The increased usage of A-EJ seen in B-cells from CdLS individuals also raises the question of what the pathological consequences of pathway choice and disturbed end resection could be. Interestingly, CdLS cells show increased number of chromosomal rearrangements that potentially could reflect a DSB repair deficiency causing an unbalanced end-resection (DeScipio, Kaur et al. 2005). In

addition, chromosome translocations are frequently found in certain lymphomas and epithelial cancers which implies that controlling the balance of DNA resection is critical to prevent toxic chromosome rearrangements (Brenner and Chinnaiyan 2009) (Nussenzweig and Nussenzweig 2010). It is also worth speculating on how and to what extent NIPBL is contributing to pathway choice. Peter's group suggests that NIPBL/cohesin has a role in recruiting 53BP1 to DSBs. In line with their observation, we find a significant decrease in 53BP1 foci formation at 30 minutes after  $\gamma$ -IR. However, this difference was not observed 90 minutes after damage induction, suggesting that the early DNA damage response is potentially defective. To understand the relevance of a 30 minutes delay in 53BP1 foci formation, it would be interesting to analyze whether CdLS/NIPBL deficient cells in fact display increased DNA resection.

By analyzing the role of DNA polymerase  $\eta$  in DI cohesion (**paper II**) we discovered that establishment of DI cohesion at the vicinity of a DSB and on undamaged chromosomes are regulated differently. We conclude this based on our finding that Pol $\eta$  is required for genome-wide DI cohesion while it is dispensable for DSB-proximal cohesion. Interestingly, we found that genome-wide DI cohesion is not required for repair of DSBs. So what is the function of genome-wide DI cohesion? We provide results suggesting that it is required for cell survival following repeated DSB induction, and we present data implying that the poorer survival capacity in these cells could be due to chromosomal missegregation. Taken together, our results suggest that S phase cohesion is not sufficient for correct chromosome segregation in the presence of DNA damage. This observation is in line with a recently published report by Aragón's group, where they showed that S phase cohesion was not sufficient to maintain cohesion after DSB induction in G2 phase (McAleenan, Clemente-Blanco et al. 2013). An interesting challenge for the future is to comprehend the role of genome-wide DI cohesion. Despite not being required for DSB repair, genome-wide DI cohesion has its own specific regulation. This highlights its important but so far mechanistically unknown role in genome integrity.

Another task for the future is to provide insight in to whether DI cohesion is an evolutionary conserved mechanism. Data providing direct evidence of a DI cohesion mechanism in higher eukaryotes are sparse. A few lines of indirect evidence exist, for instance, ChIP-seq in human cells has revealed that IR triggers an ESCO1-dependent increase in SMC3 acetylation and a reinforcement of cohesin binding (Kim, Li et al. 2010).

In **paper III**, we further studied the role of Pol $\eta$  in genome-wide DI cohesion, where we explored the connection between Pol $\eta$  and Eco1 acetyltransferase. Based on *in vitro* experiments we found that Pol $\eta$  is recognized by Eco1 as a substrate. When mutating one of these sites found to be acetylated by Eco1 *in vitro* (Pol $\eta$ -K546), Pol $\eta$  becomes dysfunctional in genome-wide DI cohesion, suggesting that it is potentially through Eco1-dependent acetylation that Pol $\eta$  functions in DI cohesion. So what is the function of Pol $\eta$  in genome-wide DI cohesion? It is interesting to speculate over how a DNA polymerase will function independent of its polymerase activity. Pol $\eta$  may represent one of many multifunctional proteins that are regulated by interplay between different post-translational modifications.

Cohesin has multiple critical roles in the DNA damage response of organisms from yeast to man. Still, many outstanding questions remain in this area and only future will tell how extensive the role of cohesin is for maintaining genome integrity.

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